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COMPLEX AND CHEMICALLY DEFINED MEDIA FOR THE GROWTH OF CRYPTOCOCCUS NEOFORMANS

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MAY 1965

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
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ABSTRACT

A maltose, ammonium acetate, inorganic salts medium containing thiamine and glutamate was formulated for growth of Cryptococcus neoformans; from which growth levels of approximately 10^9 cells per ml could be obtained within 72 to 96 hours incubation. A complex medium (nutrient broth with maltose and thiamine) with comparable growth characteristics, was also recommended. Both media supported very well the growth of 11 of 13 strains tested.

COMPLEX AND CHEMICALLY DEFINED MEDIA FOR THE GROWTH OF CRYPTOCOCCUS NEOFORMANS

Previous studies on the growth of Cryptococcus neoformans characterized the carbon and nitrogen assimilation spectra of the organism, its temperature tolerance, its aerobic nature, and its requirement for thiamine.[1-4] The majority of those studies employed solid media. In work employing liquid media, viable cell yields, when reported, have been low ($<1 \times 10^7$/ml) even with extended incubation periods of 7 to 8 days. This manuscript describes chemically defined and complex media yielding viable counts in excess of 1×10^9/ml after 48 to 72 hours' incubation. The recommended compositions of the media are given in Table 1.

TABLE 1. RECOMMENDED MEDIA FOR GROWTH OF CRYPTOCOCCUS NEOFORMANS[a/]

Constituent	Concentration, grams per liter
Complex Medium	
Maltose	40
Bacto-Peptone	5
Bacto-Beef Extract	3
Thiamine·HCl	100 µg/ml
Chemically Defined Medium	
Maltose	40
Ammonium Acetate	2
Sodium Glutamate	2
$MgSO_4 \cdot 7H_2O$	1.97
$ZnSO_4 \cdot 7H_2O$	0.0176
KH_2PO_4	2.04
K_2HPO_4	2.61
Thiamine·HCl	1 µg/ml

a. Initial pH: approximately 6.8 for each medium.

Results of growth studies employing complex medium are summarized in Table 2. Initial experiments using strain C-1, with a basal medium (0.5% peptone, 0.3% beef extract, 2% glucose, and thiamine·HCl), confirmed the requirements for aeration (shaken cultures) and thiamine (100 μg/ml). Inoculum size, over a range of 3×10^4 to 220×10^4 cells per 50 ml of medium, was not critical for maximum growth. Moreover, significantly higher cell yields were obtained at 25 C than at 34 C. Examination of various carbon sources established maltose (4%) as the best substrate, yielding approximately 1×10^9 cells per ml. The maximum growth was limited (30% less) by buffering the medium (pH 7.0); the culture pH at optimal growth was approximately 4.0.

Results of growth studies employing chemically defined medium are also shown in Table 2. Initial growth studies (strain C-1) compared various combinations of the constituents of the ammonium sulfate medium of Littman[5] and the ammonium acetate medium of Roessler et al.[6] The addition of thiamine and/or sodium glutamate (at the concentrations specified in the ammonium sulfate medium) to the ammonium acetate medium resulted in equal or better growth (approximately 2×10^8 cells per ml). The substitution of maltose for glucose in the ammonium acetate medium increased the growth level to 6×10^8 cells per ml. Supplements of the calcium, iron, manganese, or molybdenum salts to Littman's medium failed to improve the ammonium acetate medium. The substitution of ammonium chloride or ammonium sulfate, either singly or in combination, resulted in only 30 to 50% of the growth obtained with ammonium acetate.

Using 4% maltose as the energy source and decreasing the ammonium acetate level from 0.6% to 0.2% resulted in growth in excess of 1×10^9 cells per ml (at 48 hours' incubation). Increasing the level of sodium glutamate to 1 to 2% resulted in a slight increase in growth, but even at the 4 to 6% level, glutamate did not serve as a sole source of energy in the absence of maltose. One μg/ml of thiamine was sufficient for optimal growth. The original levels of magnesium and zinc in the ammonium acetate medium (0.2% and 0.00176%, respectively) were optimal for highest growth. Deletion experiments with the complete medium showed no decrease in growth at one week by omission of sodium glutamate, zinc sulfate, or dipotassium phosphate, but 20 to 30% growth reduction occurred during earlier periods of incubation (48 to 72 hr).

Growth of 13 strains of C. neoformans, in the chemically defined and complex media, is shown in Table 2. The majority of these strains (8 of 13) demonstrated higher growth in the chemically defined than in the complex medium. Considering both media, growth of 12 of the 13 strains approached 10^9 cells per ml or better.

TABLE 2. VIABLE CELL YIELDS OF VARIOUS STRAINS OF C. NEOFORMANS IN COMPLEX AND CHEMICALLY DEFINED MEDIA[a]

Strain	Complex Medium		Chemically Defined Medium	
	48 hr	72 hr	48 hr	72 hr
C-1[b]	87	103	121	128
Scott[c]	69	75	59	84
Kresge[c]	86	80	65	74
Gibson[c]	93	83	120	108
Green[c]	-[e]	31	-	3
Tucker[c]	74	72	72	86
Bishop[c]	68	65	88	120
Stanford[c]	140	158	207	232
Stokes[c]	96	88	105	116
32[d]	-	101	-	6
34[d]	-	69	-	56
35[d]	-	128	-	89
39[d]	-	58	-	85

a. Mean values of duplicate cultures expressed as 10^7 viable cells per ml.
b. From Dr. Libero Ajello, CDC, Atlanta, Georgia.
c. From Dr. C.W. Emmons, NIH, Bethesda, Md.
d. From Dr. Milton Huppert, VA Hospital, San Fernando, Calif.
e. Not counted.

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